A STUDY OF LATENT AND RECURRENT MALARIAL INFECTION AND THE SIGNIFICANCE OF INTRA-CORPUSCULAR CONJUGATION IN THE MALARIAL PLASMODIA.*

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INTRODUCTION.

IN previous contributions [1, 2, 3, 4] I have considered the subjects of latent malarial infection and of the conjugation of the malarial plasmodia. In the present communication† it is my purpose to give in detail the data which I have collected to date regarding latent infection and recurrent infection, and to consider especially the significance of intra-corpuscular conjugation as it occurs in the malarial plasmodia.

The material upon which the observations and conclusions detailed in this repor are based consisted of 1,653 cases of malarial infection, 1,267 of which were observed at the U. S. Army General Hospital, Presidio of San Francisco, in American soldiers returning from the Philippine Islands, and 386 cases studied at Camp Stotsenburg, Pampanga Province, Philippine Islands. Of the latter cases 248 occurred in Americans, and 138 in Filipinos. Besides the cases mentioned a considerable portion of the data regarding latency and recurrence has been obtained from the observation of malarial infections contracted by American soldiers while in Cuba, and studied at the Simpson U. S. Army General Hospital, Fortress Monroe, Virginia, and at Camp Columbia, near Havana, Cuba.

A word as to the classification of malarial infections: Almost all authorities now admit the existence of three species of malarial plasmodia, the tertian, quartan, and estivo-autumnal. By many the estivo-autumnal plasmodia are divided into two varieties, the tertian and quotidian estivo-autumnal plasmodia, while by some the latter organism is subdivided into a pigmented and unpigmented quotidian. Other observers maintain that there is but one estivo-autumnal parasite, the variations observed in the temperature curve being due to variations in the time of the developmental cycle, which may be 24 or 48 hours in length. Personally I have adopted the classification of Marchiafava and Bignami[5], who divide the estivo-autumnal plasmodia into two varieties, the tertian and quotidian.

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The careful study of many hundred cases of estivo-autumnal malaria has conclusively proven to my mind the existence of a tertian and quotidian estivo-autumnal plasmodium. They can be differentiated morphologically, and the infections produced by them are, when uncomplicated, clinically distinct. Anyone who can differentiate the tertian and quartan plasmodia morphologically should have no trouble in distinguishing between the tertian and quotidian estivo-autumnal plasmodia if blood from the spleen, obtained by puncture, be examined. To one who has had the opportunity of studying a large number of cases of estivo-autumnal infection the two organisms can easily be distinguished by the forms occurring in the peripheral blood.

As regards the argument that the tertian and quotidian temperatures observed in estivo-autumnal malaria are due to the sporulation of a parasite which at one time completes its developmental cycle in the blood in 24 hours and at another time in 48 hours, I can only say that it is illogical and can as well be used in the case of the tertian and quartan plasmodia by those who still maintain that there is but one species of malarial plasmodium.

I have not been able to demonstrate the occurrence of a pigmented and unpigmented quotidian estivo-autumnal parasite. While it is not uncommon to observe only unpigmented quotidian plasmodia in the peripheral blood in estivo-autumnal infections, blood obtained by splenic puncture in such cases has always shown pigmented organisms, and I have yet to observe a case in which both the peripheral and splenic blood showed only unpigmented malarial plasmodia.

1. LATENT MALARIAL INFECTIONS.

By a latent malarial infection I understand one in which the plasmodia of malaria may be demonstrated to be present in the blood of an individual, but in which no clinical symptoms of the disease of sufficient gravity to attract attention are to be observed. The term should not be confined to those instances in which no symptoms of malaria have ever been present, for if the parasites be present in the blood in recurrent cases, between the attacks the disease is as truly latent as it may be before the initial one. In many latent infections some other disease may be present, and this is almost invariably true of latent malarial infections discovered in hospital practice. It is obvious that all cases of latent malarial infection are a great source of danger to others in localities where *Anopheles* mosquitoes are present.

Of the 1,653 cases of malarial infection upon which the statistics of this contribution are based, 424, or a little over 25 per cent, were latent infections. Of these 307 were in American soldiers or civilians, while 115 occurred in natives of the Philippine Islands.

As regards the species of plasmodium present, the 422 cases were divided as follows:

Tertian	110
Quartan	8
Estivo-autumnal—	
Tertian estivo-autumnal	272
Quotidian estivo-autumnal	25
Combined tertian and tertian estivo-autumnal	7
Combined tertian and quotidian estivo-autumnal	2
Total	424

In order to understand the significance of the above table it will be necessary to consider the latent infections of Americans and of natives of the Philippines separately.

Latent infections in Americans.—The latent infections in Americans, as regards the type of plasmodium present, were divided as follows:

Tertian	81
Quartan	0
Estivo-autumnal—	
Tertian estivo-autumnal	199
Quotidian estivo-autumnal	21
Combined tertian and tertian estivo-autumnal	4
Combined tertian and quotidian estivo-autumnal	2
Total	307

The 307 latent cases observed in Americans occurred, with but few exceptions, in soldiers invalided home from the Philippines. As is evident, the estivo-autumnal plasmodia are much more frequently encountered in these cases than any other species, 220 of the 307 latent infections in Americans being due to either the tertian or quotidian estivo-autumnal parasite. The occurrence of so large a proportion of estivo-autumnal infection is not due to any peculiarity in the type of plasmodia other than that the estivo-autumnal organisms are much more resistant to quinine than either the benign tertian or quartan, and for that reason more latent infections with these types of plasmodia are found. It is also undoubtedly true that in the Philippines, as in other tropical countries, the estivo-autumnal malarial fevers are more prevalent than tertian or quartan fevers.

The fact that estivo-autumnal infections are particularly apt to exist in a latent form is of great importance, as the diagnosis of such an infection may prevent a sudden pernicious attack; and it is also

important from an epidemiological standpoint, as an individual harboring the plasmodia is, or may become, a source of infection to the community in which he is living. It will thus be seen that the blood of every individual returning from a malarial region should be examined, both as a safeguard to himself and to the community in which he resides.

Frequency of latent infection in Americans.—Of the 1,297 cases of malaria observed in Americans 307 or nearly 24 per cent were latent infections. It should be remembered that in all of these cases there were absolutely no clinical symptoms of malaria present, and had it not been for the examination of the blood these men might have been sources of infection for weeks or months.

As showing the frequency of latent malarial infections and the importance of their recognition, I quote the following from a previous report upon this subject:

"In August, 1902, Company H, Sixteenth Infantry, U. S. Army, returned to San Francisco from the Philippines, having served in the Cagayan Valley, a notoriously malarial region in those islands. On August 16, 1902, this company, out of a total strength of some 60 men, had 14 men in hospital suffering from malarial infection, all having had chills since arrival in the United States. On account of this large proportion of infected men I believed that it would be advisable to make a blood examination of the entire company, and accordingly, on August 17, I examined the blood of every man in Company H, with the following results. Of the 47 men who were doing duty, including officers, I found that 27 presented some form of malarial parasite in their blood. Of these 27 cases 25 were infected with estivo-autumnal plasmodia; 13 showing crescents of the tertian estivo-autumnal type; 2 showing crescents and ring-forms of the quotidian estivo-autuminal type; and 10 showing crescents and ring-forms of the tertian estivo-autumnal parasite. Two of the cases showed nearly full-grown forms of the benign tertian plasmodium. Thus of a total strength of 60 men, 41, which includes those in hospital, showed some form of malarial infection, while 27, without presenting any symptoms of malarial infection, showed parasites in the blood."

A study of the blood findings in this company is of great significance, not only as showing the importance of a blood examination in persons returning from the Tropics, but because, as will be noticed, the species of plasmodium present most frequently is the most dangerous of the plasmodia, and because, in most instances, the crescent form is the one present. This form, as is well known, is intended to complete its development in the mosquito and thus these men would be able to infect mosquitoes of the genus *Anopheles* wherever they might go. In this way localities might be newly infected with this most dreaded form of malarial infection.

Latent malarial infection complicating other diseases.—A very large number of latent malarial infections in Americans occur as complications of other disease processes, and it is most important that the malarial factor in such cases be recognized and removed.

As an illustration of the frequency with which latent malaria complicates other diseases I have compiled the following table, giving the original diagnosis in 106 cases in which a latent malarial infection was found to exist. In nearly all of these cases treatment with quinine, by removing the malarial infection, resulted in very marked improvement.

TABLE 1.

Diagnosis	No. of Cases	Diagnosis	No. of Cases
Chronic dysentery	15	Acute dementia	2
Chronic diarrhea	20	Convalescent from operation	1
Pulmonary tuberculosis	3	Arthritis deformans	1
Fractures and wounds	11	Retinitis	1
Chronic gastritis	8	Varicocele	1
Amebic dysentery	15	Tachycardia	1
Chronic indigestion	3	Uncinariasis	1
Hernia	3	Diabetes mellitus	1
Otitis media	3	Paraplegia	1
Acute melancholia	3	Acute endocarditis	1
Rheumatism	3	Hemorrhoids	1
Syphilis	2	Adenitis, cervical	1
Insanity	2		
Paralysis	2	Total	106

A consideration of some of the data given in the above table is of interest.

In 15 cases of chronic dysentery, non-amebic in type, a latent malarial infection was discovered. All of these patients gave a history of having suffered from malaria in the Philippines, but as not having had any symptoms of the disease since reaching the United States. The much-controverted statement that there exists a form of dysentery due to the localization of the malarial plasmodia in the capillaries of the intestine is of interest in connection with these cases, as treatment of the malarial infection with quinine resulted in every case in marked improvement and ultimately in recovery. From personal observation I am convinced that not infrequently a bloody diarrhea accompanies certain estivo-autumnal infections, when acute, and it is not at all improbable that cases may occur of chronic dysentery (so called) due to the invasion of the intestine by the plasmodia or to the action of a malarial toxin. The same remarks apply to the 20 cases of chronic diarrhea in which a coincident latent malarial infection was demonstrated.

In 15 cases of amebic dysentery a latent malarial infection was observed. In these cases *Entameba dysenteriae* was present in the feces and one of the species of plasmodia in the blood.

In nine cases of fracture and two of bolo wounds an examination of the blood showed the presence of malarial parasites, although no symptoms of infection were noticeable. This was also true in one case convalescent from an operation.

Latent infection in natives of the Philippine Islands.—The observations herein noted upon latent malarial infections in natives of the Philippines were made at Camp Stotsenburg, Pampanga Province, during a five-months' tour of duty there.

The subject of latent malarial infection in certain native races has been studied by many observers, notably Koch, Stephens and Christophers, and Annett, Dutton, and Elliot, in Africa, and James, in India.

Koch,[6] in West Africa, found that 100 per cent of native children under two years of age showed malarial parasites in their blood, and that the percentage of malarial infections decreased with advancing age; thus, children from two to five years of age showed 46 per cent infected and from five to 10 years of age, 23.5 per cent, while those over 10 years old were free from infection.

Stephens and Christophers,[7] at Accra, West Africa, found from 23 to 90 per cent of babies infected; up to eight years of age, 20 to 57 per cent; up to 12 years of age, 28 to 30 per cent, and after the twelfth year infection was found to be rare. At Lagos, of children under two years of age, 50 to 100 per cent were infected; from two to five years, 40 to 75 per cent, and from five to 10 years, 25 per cent. Annett, Dutton, and Elliot,[8] working in Nigeria, obtained the results given in the following tabulation.

TABLE 2.

Age of Child	Number Infected
0 to 1 year	27.3 per cent
1 " 2 years	63.0 "
2 " 3 "	63.0 "
3 " 4 "	51.0 "
4 " 5 "	48.8 "
5 " 6 "	34.8 "
6 " 7 "	6.6 "
7 " 8 "	27.5 "
8 " 9 "	25.0 "
9 " 10 "	14.0 "
10 years and above,	10.0 "

Averages: 0 to 5 years, 51.8 per cent
5 to 10 years, 25.0 "
10 years 10.0 "

James[9] in India found that the percentage of malarial infection among native children varied very greatly in different localities, being zero in some places and as high as 86 per cent in others. Thus at Mian Mir, children up to three years of age showed 80 per cent infected; up to five years, 66 per cent; up to 10 years, 50 per cent, while from 10 years upward none were found infected. At Ennur, children up to three years of age showed 65 per cent infected; up to five years, 51 per cent; up to 10 years, 46 per cent, and up to 15 years, 16 per cent. He found no infection in natives over 15 years of age. He also states that in the most malarious localities the immunity of the adult is very apparent, but that in those localities in which the malarial ratio is low an immunity of the adult does not appear to be established. At the time of my observations at Camp Stotsenburg the conditions present were briefly as follows:

The post is situated upon the western border of the great Pampangan plain, in the foothills of the Zambales mountains, at a considerable elevation. The soil is of volcanic nature and becomes dry, even after the heaviest rains, in a few hours. So far as I could ascertain there were no breeding-places of *Anopheles* within one mile of the post, and as these insects were at all times present, it will be seen that the prevalent idea that a mosquito will fly but a short distance in search of food is disproved in this instance. There is a considerable area of jungle country about the station, some of it within a radius of a few hundred yards; but I failed, after the most careful exploration of these jungles, to find any breeding-places of *Anopheles* nearer to the post than one mile. It was invariably found that when the grass about the post was allowed to grow to any great length, mosquitoes and malaria increased; on the other hand, when it was cut, both mosquitoes and malaria diminished very appreciably. During the latter portion of the rainy season the *Anopheles* begin to appear in great numbers, and reach their maximum during the months of November and December. This coincides with the increase in malarial infections at this post, as is shown in the following table:

TABLE 3.

Month	No. Infections	Month	No. Infections
1904		1905—*Continued*	
August	24	April	10
September	57	May	14
October	24	June	17
November	58	July	11
December	75	August	29
1905		September	31
January	76	October	43
February	27	November	54
March	20	December	126

During the five months I was stationed at Camp Stotsenburg I observed 386 cases of malaria in which I was able to demonstrate the parasite in the blood. Of these, 248 occurred in Americans and 138 in natives. As regards the type of infection, 98 were infected with the tertian plasmodium, of which 63 were Americans and 35 natives; eight with the quartan, of which two were Americans and six natives; and 272 with the estivo-autumnal plasmodia, 183 being Americans and 89 natives. Of the estivo-autumnal infections, 258 were due to the tertian estivo-autumnal plasmodium and 14 to the quotidian variety. There were eight combined infections with the tertian and the estivo-autumnal tertian plasmodium. Of these cases there were 227 initial infections, all in Americans; 38 recurrent infections; in Americans, 18, and in natives, 20; and 115 latent infections, all in natives.

It appeared probable to me in considering the malarial situation at this post that the natives living in the barrios in close proximity were in all likelihood the principal source of infection, for while, as has been stated, the sanitary conditions in the post proper were such as to prohibit the belief that much malarial infection could originate there, breeding-places of mosquitoes abounded in the barrios and mosquitoes of the genus *Anopheles* were much more

numerous in them than at the post. The barrios were constantly visited by the soldiers, especially at night, and in them the conditions for the spread of both mosquito and human infection were ideal. In order to determine how large a percentage of the native population of these barrios was infected, I made blood examinations, whenever they could be obtained, of the natives living within two miles of the post. The results proved beyond question that the origin of malarial infection at Camp Stotsenburg was to be found very largely in the natives being in the immediate vicinity, and that any efforts to limit the disease must take this condition into account. In a considerable number of the cases of latent infection, even in the youngest children, a history of previous attacks of fever could be obtained, but in none of them were any symptoms of malaria observed at the time of examination. In all, the blood of 225 natives was examined and 115, or 51.1 per cent, were found to be infected. This percentage of latent malarial infections should not be regarded as typical of all localities in the Philippines, for many localities are free from malaria, while in others the malarial index is low. Routine blood examinations of the natives will give much valuable information as to the endemicity of malarial infection in these islands, and such examinations should be made before permanent military posts or residences are established in the Tropics.

Of the 115 infections found, the tertian plasmodium was present in 29, the quartan in six, and the estivo-autumnal in 77; of the latter, 73 were due to the tertian estivo-autumnal plasmodium and four to the quotidian. There were three infections with the tertian and the tertian-estivo plasmodium.

Latent infection in the adult native.—I was able to examine the blood of but 45 adults, of which 28, or 62.2 per cent, were infected; of these, five were due to the tertian plasmodium and 23 to the tertian estivo-autumnal plasmodium. It is very probable that a further study of the blood of a larger number of adults would materially reduce this great percentage of infections, but, from the results obtained, it is evident that the adult Filipino is more often infected than the negro in Africa or the natives of India.

Both Koch in Africa and James in India, found that the percentage of infections in adult natives is very small. In the planting districts of the Duaro, James found no adults with plasmodia in their blood, although 65 to 75 per cent of the children were found to be infected. It is probable that the adult Filipino possesses little or no immunity to malaria, despite the fact that in malarial localities such adults have from

childhood suffered from repeated attacks of the disease. I have notes on several native adults who, within two years, were admitted to the hospital from 8 to 16 times with malarial infection.

This lack of immunity to malaria in the adult native of this country appears to me to be a very significant and important economical fact, for it is invariably true that a people suffering from long-continued malarial infection are poor producers, especially along agricultural lines, where strength and endurance are demanded.

Latent infection in native children.—Of the 180 children whose blood was examined, 87, or 48.3 per cent, showed the presence of malarial plasmodia. Of these infections 34 were due to the tertian plasmodium, six to the quartan, and 44 to the estivo-autumnal variety; of the latter 40 were infected with the tertian estivo-autumnal plasmodium and four with the quotidian estivo-autumnal plasmodium. There were three infections combined with the tertian and the tertian estivo-autumnal plasmodium.

The infections in children diminished in number with advancing age; thus, between the ages of one month and five years, among 40 children, 79 per cent were infected; between five and 10 years, 37 per cent; and between 10 and 15 years 24.5 per cent. These results agree with those of Koch, Stephens and Christophers, James and others, who invariably found that the younger the child, the more susceptible it was to malaria.

The following table illustrates the relationship between malarial infection and the age of the individual, as observed in the barrios about Camp Stotsenburg; it gives the number examined, the number attacked in five-year periods, the percentage of those infected, and the type of infection.

TABLE 4.

Age	No. infected	Per Cent Infected	Tertian	Quartan	Estivo-Autumnal
1 to 5 years—40 children.....	30	72.5	10	4	16
5 to 10 " —54 "	20	37.0	8	1	10
10 to 15 " —53 "	13	24.5	5	1	7

Only 147 children are considered in the above table, as only in that number could the age be ascertained with certainty.

TABLE 5.

Barrio	Age	No. Examined*	No. Infected	Per Cent Infected
A	1 to 5 years	6	5	83.3
	5 to 10 "	5	3	60.0
	10 to 15 "	6	2	33.3
	Over 15 "	5	3	60.0
B	1 to 5 years	10	8	80.0
	5 to 10 "	7	4	57.1
	10 to 15 "	6	2	33.3
	Over 15 "	20	10	50.0
C.....	1 to 5 years	12	12	100.0
	5 to 10 "	6	5	83.3
	10 to 15 "	3	2	66.6
	Over 15 "	14	9	64.2

* In explanation of the small number of individuals examined between the various ages it should be stated that the barrios mentioned are simply collections of a few huts, each *barrio* not containing more than a dozen or so, with a total population each of from forty to sixty individuals.

Barrio infections.—It is interesting to observe how the little barrios, all situated within two miles of the post, varied as regards the number of infected individuals. The foregoing table well illustrates this point.

In the town of Mabalacat examinations were made of the school children with the following results:

Of children between the ages of five and 10 years, 25 were examined, of which seven, or 28 per cent, were infected; of children between 10 and 15 years of age, 35 were examined, of which seven, or 20 per cent, were infected.

In the town of Dolores, 11 school children between the ages of five and 10 years were examined, of which seven, or 63.6 per cent were infected, while of 13 children between 10 and 15 years of age, four or nearly 31 per cent, were infected.

In Mabalacat the children of the better class of natives attend one school while those of the lower class attend another, but the percentage of latent malarial infections was practically the same in both schools.

Family infection.—Not only do the barrios differ in the ratio of malarial individuals to those in health, but certain portions of the larger barrios are most malarious, while other portions are almost free from the infection; investigation always demonstrated that this is due entirely to the number of *Anopheles* breeding-places about such areas. Thus the malarial infections may be said to be strictly local infections, being confined within certain well-defined limits, even in infected localities.

Not only were certain portions of the barrios free from infection and badly infected in others, but it was observed that malaria was very largely a family disease, certain families suffering severely while others were free from infection. The following table illustrates the family character of malarial infection, being compiled from the data obtained in one barrio where all the families resided:

TABLE 6.

Family	No. Members	No. Infected	Variety
1.....	4	2	1 es.-autumnal; 1 tertian
2.....	3	2	2 estivo-autumnal
3.....	4	2	1 es.-autumnal; 1 tertian
4.....	5	4	2 es.-autumnal; 1 tertian 1 quartan
5.....	4	2	2 estivo-autumnal
6.....	3	2	2 estivo-autumnal
7.....	4	3	2 es.-autumnal; 1 tertian
8.....	3	2	1 es.-autumnal; 1 tertian
9.....	3	2	2 tertian
10.....	6	4	2 es.-autumnal; 2 tertian

In considering this table it should be remembered that these infections were latent in character, and I have repeatedly observed families in which every member was suffering from a malarial infection, either latent or active. Such are numbers four and 10 in the above table. Family No. 4 is of interest because, of its five members, one was suffering from a severe attack of tertian malaria at the time that I made the blood examination, while of the other four two presented estivo-autumnal, one the quartan, and the other the tertian plasmodium in the blood, so that in this one family all the varieties of the parasite could be studied.

Pathology of latent infections.—The study of the pathological lesions found in cases of latent malarial infection demonstrates that

the malarial plasmodia may unde go schizogony within the spleen without producing symptoms of the disease.

If we confine the term "latent infection" to those cases in which no symptoms are present and no parasites are found in the peripheral blood after repeated examinations, it will at once be seen why the pathology of this condition has not been thoroughly investigated. Such cases of malarial infection will only be discovered at autopsy, the patient having perished from some other disease. Outside of the Tropics and the more malarial regions of our own country, such cases are very rare. I have observed seven cases in which, at autopsy, death having been due to some other disease, latent malarial infection was discovered upon microscopical examination of the viscera. During life none of these patients presented any symptoms of malaria, nor were malarial parasites found in the blood after repeated examinations. The patients were under observation in the hospital for several weeks, were carefully studied clinically, and in none was malarial infection suspected. Of the seven cases three were benign tertian infections and four estivo-autumnal infections of the tertian type.

The pathological lesions found in the tertian cases were confined to the liver and spleen, especially the latter. The spleen was considerably enlarged, decreased in consistence, and somewhat pigmented. Microscopical examination of sections showed intense congestion of the sinuses together with pigmentation, the latter being marked along the edges of the Malpighian bodies and the fibrous trabeculae. Many of the cells of the splenic pulp were pigmented. Numerous parasite-infected red cells and pigmented leucocytes were present, and while the infected red cells were not nearly as numerous as in acute infections or as in more advanced latent infections, they were still quite numerous. The plasmodia were in about the same stage of development in the individual case, but it so happened that the patients had died at such a period that the entire cycle of the tertian parasite within the human body could be followed. As far as could be ascertained the plasmodia did not differ in their appearance from those seen within the red cells in the peripheral blood during an acute infection. The segmenting bodies were numerous in one case, the segments, in fresh smears from the spleen, appearing somewhat more refractive and clearly outlined than in the peripheral blood. The staining reactions of the plasmodia did not differ from those present in the blood.

The capillaries also contained pigmented leucocytes and macrophages, the latter containing much pigment in large blocks and often one or more parasites.

The liver, macroscopically, did not differ in appearance from that of the normal organ but upon microscopical examination the capillaries showed within them a few pigmented leucocytes, some containing what appeared to be degenerating plasmodia. No infected red cells were observed. In the estivo-autumnal infections the spleen

appeared as in the tertian infections, but the enlargement and pigmentation were not as marked. The consistence was much decreased. Upon microscopical examination the same changes were found, as in the tertian cases, the splenic sinuses being congested, the cells of the splenic pulp increased in number, considerable pigmentation was present, and the presence of infected red cells and melaniferous leucocytes was noted. The infected red cells were not as numerous as in the tertian infections. The parasites observed within the red cells were almost all in the same stage of development, but the four patients showed all stages of schizogony of the plasmodia, no single one of them, however, showing all the stages. The young forms of the estivo-autumnal parasite were similar in appearance to the young forms found in the peripheral blood, being small, hyalin rings, well defined, and presenting, in the fresh smear, marked ameboid motion of limited extent. The older parasites were round or ringlike, and contained a small amount of pigment in the form of very fine, reddish-brown granules. In one case numerous segmenting bodies were observed, the segmentation always taking place within the red cell. The segments varied in number, the largest number counted being 24, the smallest 12. No crescents were observed in any of the sections.

The pathological changes in the liver were similar to those in the liver of the tertian cases. No infected red cells were found, although a considerable number of pigmented leucocytes were observed, together with some free pigment.

The pathology of latent malarial infection may be summed up by the statement that before any clinical symptoms of infection are present the plasmodia are undergoing normal schizogony within the spleen, and can be demonstrated within that organ either by splenic puncture or at autopsy. The lesions produced are the same in character as occur in acute infections but, of course, less extensive.

Cause of latency.—Many theories have been advanced regarding the cause of latency in malarial infection, and these will be considered very briefly under the discussion of the etiology of recurrences.

Practical deductions.—From the data which have been given I believe that it is obvious that latent malarial infections are of much importance from both epidemiological and clinical standpoints; it is certainly true that latent infections are a great source of malarial disease, and in the Tropics, the latent infection of the native is undoubtedly the principal source of infection of the white man. This is well illustrated in the study of malarial infection at Camp Stotsenburg, and the same condition obtains, without doubt, in every malarial locality in these islands. The importance of an examination of the blood of the native population of any given locality cannot be overestimated in the fight against malaria. This is especially true in the Tropics, for only through the results of such examination can the endemic areas of malaria be found and guarded against. Only

to one who has attempted it can the almost hopeless task of exterminating mosquitoes in tropical regions be appreciated, and, in fact, in many such localities it is impossible of accomplishment. This being so, it is apparent that it is useless to expect to rid a locality of malaria from which the mosquitoes cannot be eliminated, unless the infection is first stopped among the natives; and in the Tropics it appears to me that the greatest hope of success in combating malaria lies in the distribution of quinine to the infected native. It would then make no difference how numerous *Anopheles* might be, they would be harmless if the native were rendered free from the plasmodia by the use of quinine.

2. RECURRENT MALARIAL INFECTION.

It is probably a fact that with very few exceptions (and these most vigorously treated with quinine), every primary malarial attack is followed by one or more relapses or recurrences. By recurrences I mean the appearance of symptoms due to the same group of parasites that caused the original infection, and not a reinfection by another group. So common are recurrences in malaria that a prevalent belief is that "once a victim of malaria, always a sufferer from the disease;" while this belief is unfounded, recurrences often do persist for months, and sometimes, although very rarely, for years. They are most common and persistent in the estivo-autumnal infections, as would be expected from the greater resistance of these infections to treatment.

To the clinician the time elapsing between the initial attack and the recurrences is of greatest interest, while to the investigator the etiology of recurrences and the *modus operandi* have proven a fruitful field for contoversy, and, from the very nature of the problem, of theoretical deduction.

Time of recurrence.—Authorities differ somewhat as to the time of recurrence in the different types of malarial infection, and when we consider the difficulty of ruling out reinfections, especially in those who reside in a malarial locality, as well as the influence which treatment has in delaying recurrence, the slight difference in the time as given by various observers is indeed surprising. In malarious regions it is obviously impossible, in many instances, to be sure that

the reappearance of symptoms is not due to a reinfection, unless a different type of parasite be present than that found during the initial attack. As a basis for the computation of the time of recurrence Celli[10] reckons "as recurrent every case of fever which repeats itself in the same individual from the July of one year to the end of June of the following year, or during all the cycle of the same yearly epidemic." While, of course, errors are bound to occur with this method of computing recurrences, Celli believes, and I think justly, that for practical purposes it is as perfect as is possible under the circumstances. I have used this method in compiling the table of recurrences which follows, but have taken the further precaution of selecting only those cases which I am reasonably sure could not have become reinfected.

Borius,[11] an investigator working in Senegal, found that recurrences took place preferably upon the 7th, 14th, 21st, and 28th days after the initial attack. Of 226 cases, 128 relapsed, and of these 18 relapsed upon the 7th day, 64 on the 14th; 31 on the 21st; and 9 on the 28th day. Ninety-eight cases relapsed irregularly on the 9th, 10th, 16th, and 20th days. All of the cases took quinine at the time of the attack. Barudel[12] from his observations, concludes that quotidian fever most frequently relapses upon the 7th day, tertian upon the 14th, and quartan upon the 20th. Mariotti-Bianchi[13] concludes that benign tertian infections relapse between the 5th and 18th days after infection, while estivo-autumnal relapses between 5 and 21 days, but frequently between 5 and 9 days; Ziemann[14] found that in West Africa estivo-autumnal infections relapsed between 9 and 12 days after the primary attack, which, as he points out, is practically the same as the incubation period. Werlhof,[15] from his experience decides that tertian infections relapse most frequently in the second, and quartan in the third week, while Duden[16] claims that on the east coast of Africa, quotidian fever relapses almost invariably upon the seventh day.

Authentic recurrences after long intervals of time are rare, but unquestionably occur. Thayer[17] relates an interesting example and Mariotti-Bianchi observed in tertian infections recurrences between three and 13 months and in estivo-autumnal fevers, between four and eight months apart. V. Leyden[18] observed a recurrence of a malarial infection three years after the initial attack, and Schilling[19] has observed recurrences after eight and one-half months and after two and one-half years. As regards recurrences after such long intervals as two or three years I agree with Mannaberg[20] that the evidence is not sufficient to prove undeniably that the so-called recurrences were not reinfections and such cases appear very doubtful.

The following tables of recurrences in estivo-autumnal and tertian malaria are prepared from carefully selected cases in which reinfection was considered at least very improbable, and which I believe may be considered as portraying the exact length of time occurring between the relapses in these types of the malarial fevers.

The patients were all American soldiers observed in hospital and treated thoroughly with quinine during the active symptoms, while most of them received prophylactic doses of the drug once a week between the relapses, which undoubtedly delayed them somewhat.

TABLE 7.

ESTIVO-AUTUMNAL TERTIAN RECURRENCES.—TIME OF THE VARIOUS RECURRENCES IN 55 CASES OF TERTIAN ESTIVO-AUTUMNAL INFECTION.

Case No.	Date of Initial Attack	First Recurrence	Second Recurrence	Third Recurrence	Fourth Recurrence	Fifth Recurrence
1	Oct. 12	10 days	30 days	36 days	.....	.....
2	Nov. 19	12 "	.....	.....	.....	.....
3	Feb. 27	15 "	20 days	30 days	.....	.....
4	Nov. 2	18 "	30 "	30 "	.....	.....
5	Mar. 30	19 "	20 "	.....	.....	.....
6	Dec. 8	19 "	.....	.....	.....	.....
7	Jan. 24	20 "	.....	.....	.....	.....
8	Feb. 12	20 "	60 days	.....	.....	.....
9	Dec. 24	20 "	.....	.....	.....	.....
10	Feb. 6	20 "	20 days	.....	.....	.....
11	Feb. 6	20 "	48 "	.....	.....	.....
12	Dec. 25	21 "	33 "	.....	.....	.....
13	Mar. 1	22 "	.....	.....	.....	.....
14	Nov. 29	22 "	.....	.....	.....	.....
15	Nov. 14	24 "	.....	.....	.....	.....
16	Feb. 4	24 "	20 days	38 days	30 days	.....
*16½	Oct. 30	24 "	16 "	.....	.....	.....
17	Aug. 29	24 "	26 "	.....	.....	.....
18	Mar. 17	24 "	.....	.....	.....	.....
19	Feb. 4	25 "	16 days	20 days	.....	.....
20	Dec. 30	26 "	36 "	30 "	90 days	30 days
21	Jan. 26	26 "	48 "	90 "	.....	.....
22	Jan. 11	26 "	22 "	.....	.....	.....
23	Oct. 2	27 "	.....	.....	.....	.....
24	Nov. 2	27 "	.....	.....	.....	.....
25	Mar. 2	27 "	52 days	.....	.....	.....
26	Feb. 5	28 "	21 "	20 days	21 days	.....
27	Dec. 12	28 "	28 "	.....	.....	.....
28	Oct. 29	29 "	48 "	15 days	.....	.....
29	Jan. 17	30 "	.....	.....	.....	.....
30	Jan. 1	30 "	30 days	.....	.....	.....
31	Jan. 19	30 "	.....	.....	.....	.....
32	Jan. 20	32 "	.....	.....	.....	.....
33	Oct. 19	33 "	26 days	90 days	.....	.....
34	Jan. 19	34 "	40 "	.....	.....	.....
35	Oct. 18	34 "	50 "	.....	.....	.....
36	Jan. 25	34 "	26 "	17 days	.....	.....
37	Oct. 21	36 "	56 "	.....	.....	.....
38	Feb. 30	36 "	66 "	.....	.....	.....
39	Aug. 13	36 "	35 "	.....	.....	.....
40	Nov. 27	36 "	.....	.....	.....	.....
41	Sept. 1	37 "	49 days	.....	.....	.....
42	Oct. 18	38 "	.....	.....	.....	.....
43	Oct. 17	38 "	.....	.....	.....	.....
44	Aug. 13	38 "	.....	.....	.....	.....
45	Sept. 6	41 "	.....	.....	.....	.....
46	Oct. 31	42 "	20 days	.....	.....	.....
47	Jan. 1	45 "	30 "	.....	.....	.....
48	Nov. 3	46 "	21 "	.....	.....	.....
49	Dec. 7	49 "	.....	.....	.....	.....
50	Feb. 24	50 "	24 days	41 days	.....	.....
51	Oct. 24	51 "	39 "	.....	.....	.....
52	Jan. 18	61 "	156 "	.....	.....	.....
53	June 14	64 "	66 "	14 days	20 days	20 days
54	Mar. 3	80 "	120 "	96 "	.....	.....

*The case numbered 16½ brings the total of the table to 55 cases.

A consideration of the table shows that the first relapse in estivo-autumnal tertian infection occurred at periods varying from 10 up to 80 days; in none of the cases did a

relapse occur before 10 days after the initial infection, which does not agree with the results obtained by Mariotti Bianchi and Ziemann, already mentioned, who found that this type of malarial infection relapsed most frequently in from 5 to 20, and 9 to 12 days respectively. The administration of quinine probably delayed the relapse in my cases, but even so, it will be noticed that the vast majority of the cases did not recur until after the 24th day. Taken in periods of 10 days, an analysis of the table shows that six cases recurred between the 10th and the 20th days; 23 between the 20th and 30th days; 16 between the 30th and 40th days; five between the 40th and 50th days; and the remainder at periods later than the 50th day. The greatest number of relapses occurred between the 20th and 30th days, namely, 23, and almost as many between the 30th and 40th days, namely 16. Of single days five cases recurred upon the 20th day; five upon the 24th day; four upon the 36th day, and three upon the 26th, 27th, 30th, 34th, and 38th days.

In most instances secondary relapses occurred at longer intervals than the primary one, although numerous exceptions to this statement will be noticed in the table.

The statement that the longer the infection lasts the longer becomes the period between relapses is not borne out in this table, if the majority of the cases be taken into account. Thus in Case 16, relapses occurred at periods of 20, 38, and 30 days; in Case 20, at periods of 26, 36, 30, 90, and 30 days; in Case 26, at periods of 28, 21,20, and 21 days; in Case 36, at periods of 26, 34, and 17 days; in Case 50 at periods of 50, 24, and 41 days, and in Case 53, at periods of 64, 66, 14, 30, and 20 days.

Of the 55 cases, 36 had two relapses; 14, three relapses; 4, four relapses, and 1, five relapses; this well illustrates the persistency of estivo-autumnal infection, and its resistance to treatment, unless the treatment be continued for a long period of time.

A most interesting feature of the estivo-autumnal cases is the fact that 21 of them suffered from at least two relapses when the primary relapse occurred 27 days after the acute initial attack. In the benign tertian infections not a single secondary relapse occurred when the primary relapse occurred after the 26th day. In the estivo-autumnal infections it will also be noticed that no security against future recurrences is apparent when the primary relapse occurs after a long period of time from the acute attack, whereas in the benign tertian infections one can almost rest assured that secondary relapses will not occur if the primary relapse occurs a month after the acute initial attack.

Tertian recurrences.—The following table gives the data concerning recurrences in 18 cases of benign tertian malaria. The number is small, but while my records contain data covering hundreds of tertian cases, in only 18 cases can I be sure of genuine recurrence. The vast majority of tertian cases, if properly treated, do not recur, and very many of them recover spontaneously, although in such cases (which comprise, of course, only a very small number in my records of hospital cases) recurrence is much more common and, indeed, may be stated to be the rule.

A consideration of this table shows that tertian relapses occurred at periods of from 16 to 41 days; the shortest period after the acute initial attack was 16 days, while Mariotti-Bianchi found that recurrences in benign tertian appeared as early as five days and not later than 18. Of the 18 cases, relapse occurred in 1 in 16 days; in 1 in 18 days, in 1 in 19 days; in 2 in 20 days; in 3 in 21 days; in 2 in 22 days; in 1 in 27 days; in 3 in 30 days; in 1 in 33 days; in 1 in 37 days; in 1 in 38 days, and in 1 in 41 days. Secondary relapses occurred in 10 cases; a third relapse occurred in six cases; a fourth in three cases and a fifth in one case. No secondary relapse

TABLE 8.

TIME OF RECURRENCES IN 18 CASES OF TERTIAN INFECTION.

Case No.	Date of Initial Attack	First Recurrence	Second Recurrence	Third Recurrence	Fourth Recurrence	Fifth Recurrence
1	Nov. 2	20 days	21 days	.....	.....	.....
2	Aug. 4	18 "	20 "	.....	.....	.....
3	Aug. 28	19 "	30 "	26 days	46 days	.....
4	Nov. 6	20 "	24 "	.....	.....	.....
5	Jan. 17	20 "	32 "	30 days	24 days	.....
6	Nov. 23	21 "	20 "	26 "	.....	.....
7	Oct. 6	21 "	30 "	.....	.....	.....
8	Sept. 17	21 "	22 "	27 days	.....	.....
9	Aug. 27	22 "	36 "	.....	.....	.....
10	Feb. 12	22 "	18 "	16 days	27 days	30 days
11	Jan. 17	27 "	.....	.....	.....	.....
12	July 20	30 "	.....	.....	.....	.....
13	May 3	30 "	.....	.....	.....	.....
14	Nov. 1	30 "	.....	.....	.....	.....
15	Sept. 22	33 "	.....	.....	.....	.....
16	Sept. 1	37 "	.....	.....	.....	.....
17	Dec. 13	38 "	.....	.....	.....	.....
18	Sept. 22	41 "	.....	.....	.....	.....

occurred if the primary relapse occurred more than 22 days after the initial attack, and therefore, in tertian infections we may say that if a relapse does not occur within a month after the acute attack there is practically no danger of future recurrences.

A study of the two tables given enables us to conclude that in estivo-autumnal tertian infections, relapses occur most frequently between the 20th and 40th days after the initial acute attack, and in benign tertian infections between the 15th and 22d days; during these "periods of relapse," as they may be termed, quinine should be given in full therapeutic doses, and increased to the point of cinchonism at the appearance of the first symptoms indicative of a malarial attack. It should be remembered that quinine was given in prophylactic doses, once a week, to all the cases quoted in the tables, and thus the occurrence of the relapses was undoubtedly delayed somewhat, which accounts for the difference in my results and those of others who have described relapses in cases uninfluenced by quinine.

Etiology of recurrences and latency.—The etiology of recurrent and latent malarial infection should be considered together, as an explanation of the one would necessarily explain the other, since the infection must be latent in the system during the period between the recurrences. Many investigators have attempted to explain recurrence in malarial infection, but almost all of our knowledge of these subjects is theoretical and incapable of proof. It is evident that the plasmodia must exist in some form in the body during the intervals

in which no symptoms are present, and I have already shown, in considering the pathology of latent infection, that plasmodia may be demonstrated in the spleen of patients who have died from some other disease, and in whom no symptoms of malarial infection were ever present; these plasmodia did not differ in appearance from those observed in the peripheral blood in the acute malarial attacks, and, furthermore, were undergoing normal schizogony within that organ, but in numbers insufficient to produce clinical symptoms. In such cases it may be urged that after a certain period of multiplication the plasmodia become numerous enough to produce symptoms of malarial infection, and thus a relapse follows. While this is undoubtedly true in some instances, it will hardly account for long-interval relapses, for it is impossible to conceive that the plasmodia of malaria can continue to grow and multiply for weeks and months without becoming numerous enough to produce symptoms of infection.

To overcome this objection, Bignami[21] considers that the plasmodium exists in some latent form, perhaps encapsulated, in the spleen or other internal organ, which may not be rendered visible with our present staining methods, and which, resting as a spore, is only set free under certain favorable conditions, the nature of which we are ignorant of.

Celli, in discussing this subject, says: "How are these recurrences explained? It is difficult to say; perhaps they depend on forms resulting from sexual multiplication, that remain inert in some viscera—possibly the bone marrow—and, from time to time, invading anew the blood, give rise to new generations of the asexual cycle."

A. Plehn[22] interprets the basophilic granules, so commonly seen in the infected red cells in tertian malaria, as latent forms of plasmodia which multiply in the blood until conditions are favorable for the development of the large ameboid forms which produce the symptoms of infection. It is unnecessary to state that this explanation of basophilic granulation is wholly unique and untenable.

Recently Schaudinn,[23] in an excellent study of *Plasmodium vivax*, stated that recurrences are due to parthenogenesis of the marcogametes which are not fertilized by the microgametocytes; these remain in the human host and eventually liberate schizonts which penetrate the erythrocytes, undergo schizogony, and thus produce a

relapse. This process is completed in from 9 to 12 days, and, according to Ziemann and Mariotti-Bianchi, agrees with the period in which relapses most frequently occur. So far as I know Schaudinn's results have not been confirmed and it is difficult to understand how such a process explains relapses occurring at irregular intervals as shown in the tables given, where it is obvious that no regularity is present in the development of either the primary relapse or those succeeding it. How can the parthenogenesis of the macrogamete, which must occur in a cyclical manner, be the cause of relapses occurring all the way from 16 to 80 days after the initial attack of fever? For this reason, I am inclined to believe with Bignami and Celli that the cause of relapse will be found in a resistant form of the plasmodium (a latent form), which is capable of remaining unchanged within the human body for considerable periods of time, and which, under favorable conditions, undergoes further development and gives rise to the symptoms which are the evidence of a recurrence.

In considering the significance of intracorpuscular conjugation in the malarial plasmodia I shall discuss this subject further, indicating how this peculiar process may bear a very important relation to latent and recurrent malarial infection.

The significance of intracorpuscular conjugation in the malarial plasmodia.—In December, 1905, I described[24] a process of conjugation in the malarial plasmodia taking place within the infected red cells. This process had been described before by Ewing,[25] but my conclusions regarding its significance differed greatly from his, as will be seen in the following brief summary of his important work upon this subject. Ewing considered the process of rare occurrence and of comparatively little significance. In describing it he says:

> In four cases of tertian infection I have encountered appearances which seem to admit of no other explanation than that of conjugation of malarial parasites. In a considerable number of other cases similar appearances were found, but much less frequently. A great many red cells showed double infection with young rings. In many instances these rings were entirely separate, each exhibiting a singular large granule of chromatin. Many cells, however, contained two rings which were clearly fused together along one segment of the ring, and two large chromatin granules were then invariably found at different poles of the rings.

Describing what he considers as a later stage of the same process, he says:

On examining the parasites in later stages of development, most of them were found to have lost the ring form, and to have spread out into a large number of threads, with nodal thickenings, variously curled in the red cell. These threads evidently represented the pseudopodia of a very active ameboid stage. The chromatin masses were now subdivided into 10 or 12 granules, but in the majority of the cases the masses were far apart, and showed no tendency to unite. In many cells, however, the ameboid figures were less marked, and the masses of chromatin lay side by side, united by a little achromatic substance.

Regarding the significance of the process Ewing is undecided. He says:

It would seem that a process so fundamental as the conjugation of individuals, if it occurs at all, ought to be an invariable feature of every active infection, but there is not sufficient evidence upon which to base any such claim. The four cases referred to as furnishing numerous clear examples of conjugation were selected on account of the abundance of the conjugating forms, but in many other cases less numerous, though equally distinct, examples were seen, indicating that the process is of very frequent occurrence. On the other hand, it must be admitted that the majority of specimens from routine cases fail to show any distinct traces of the process; from which it may be concluded that conjugation is not an essential feature of the growth of the parasite.

In a later contribution he says:

The extent and significance of this form of conjugation it is difficult to determine. In the cases in which it can be profitably studied, parasites are very abundant, and in most cases few indications of the process can be detected. It is, therefore, probably not essential to sporulation, and when parasites are scanty the chances of finding typical examples of conjugating pairs are greatly reduced, but the peripheral blood may not be a complete index of the process in the internal viscera. It seems probable that conjugation occurs in the first generations of the infection, and becomes less frequent as the disease progresses, the infection in the human host thereby tending to limit itself.

The material for my own studies upon this subject consisted of nearly 300 cases of malaria presenting clinical symptoms, observed at the U. S. Army General Hospital at the Presidio of San Francisco, Cal., together with over 100 cases of latent infection observed at the same place, a series of 75 latent cases in native Philippine children studied at Camp Stotsenburg, and 96 cases of acute infection observed at that post and in Manila. As a result of my studies, which cover a period of over five years, I am convinced that intracorpuscular conjugation is not an accidental occurrence of no essential importance in the life-history of the malarial plasmodia, but is a process which is most essential and one that occurs invariably in all acute infections uninfluenced by quinine and also in all recurrent cases in which the infection is of sufficient strength to produce marked clinical symptoms.

Morphology of intracorpuscular conjugation.—The process may be roughly divided into three stages. In the first stage, or stage of protoplasmic union, the two young hyalin rings are in contact, and careful examination demonstrates that at the point of contact there is a direct union of the protoplasm; in stained specimens it will be noted that the chromatin of the nuclei of the two parasites is separated and that the union begins in the protoplasm of the plasmodia; in fact, it should be stated that intracorpuscular conjugation always occurs between two young hyalin ring forms and is always completed before the formation of pigment. In no case can conjugation be recognized after the formation of pigment, and the large pigmented bodies described by Ewing as conjugating bodies in which the chromatin had divided into several distinct masses are easily recognized to be normal presegmenting plasmodia, in which such a division of the chromatin is always present. In this stage the chromatin masses may be situated at any portion of the periphery of the two rings, but it is extremely rarely that they lie in apposition at this stage. I have been unable to detect any differences in the appearance of the two conjugating bodies, for while one may occasionally be a little larger than the other, this is not so as a rule, and the chromatin masses are always of the same size.

In the second stage, which may be designated as that of complete protoplasmic union, the chromatin masses become situated in the protoplasm of one organism, formed by the gradual union of the protoplasm of the two; the chromatin granules may be opposite one another or at any portion of the periphery of the plasmodia, sometimes almost in apposition. The complete union of the protoplasm of the two plasmodia results in a more or less perfect ring-shaped organism, slightly larger than either of the original parasites, containing two masses of chromatin, surrounded by achromatic substance.

The third stage of the process, or stage of chromatic union, is characterized by the union of the chromatic granules, with the apparent exclusion, in many cases, of a very minute grain of chromatin, before the union is complete.

Briefly stated, then, intracorpuscular conjugation consists in the complete and permanent union of the protoplasm and nucleus of

two young amebula within the erythrocyte. It is absolutely necessary to the maintenance of malarial infection in man, and in these instances in which it does not occur, the plasmodia undergo a sexual sporulation for a limited time and then perish, thus leading to spontaneous recovery. It is present most typically in those cases in which the clinical symptoms are most severe, and is present in all the varieties of malarial infection, although most easily observed in the estivo-autumnal infections.

Conjugation in Protozoa in general and its significance.—In order to understand the significance of intracorpuscular conjugation as seen in the malarial plasmodia, it is necessary to review briefly the phenomena and significance of conjugation as they occur in other protozoan organisms, for it is only by thus summing-up, as it were, the significance of the process in all the forms of the Protozoa in which it has been observed, that we are enabled to arrive at any conclusions regarding its significance in the plasmodia of malaria.

The process of conjugation is common among the Protozoa and may be observed in all its gradations from the union of absolutely similar organisms, in which sex cannot be distinguished, to the union of highly specialized forms in which the male and female organisms may be easily distinguished.

The Rhizopoda.—Conjugation occurs comparatively seldom in the Rhizopoda most frequently among the Amebina and Helioza. Among the Amebina conjugation is probably much more common than is generally supposed. Holman[26] observed the temporary union of a large and small ameba, resulting apparently in the production of swarm-spores in each. Penard[27] describes the union of different-sized individuals in *Amoeba spatula*, the larger absorbing the smaller, the union being permanent. I have observed conjugation in *Entamoeba coli* several times, the process consisting in the permanent fusion of apparently similar individuals, the protoplasm of the two organisms first blending, followed by the fusion of the nuclei. In *Entamoeba dysenteriae* (*histolytica*) I have observed conjugation frequently, and I believe that the process is of greater import in the life-history of these parasites than is generally thought.

Among the Heliozoa, conjugation has been certainly demonstrated in *Actinophrys sol* and in *Actinosphaerium Eichornii* by Schaudinn;[28] in the former free-swimming forms unite, coalesce, and develop a firm protective covering; the union is protoplasmic, the nuclei remaining distinct, and finally divide, thus making four nuclei; two of these fuse, and give rise by mitosis to daughter-cysts, while the remaining two degenerate and disappear. In *Actinosphaerium Eichornii* conjugation is stated to be common before reproduction commences.

The Flagellata.—Among the Flagellata conjugation is very common and may occur between similar-sized individuals, or between individuals dissimilar in appearance. The product of the conjugation or the *zygote* gives birth to the motile organisms which reproduce without conjugation. To Dallinger and Drysdale[29] we are indebted for much of our knowledge regarding conjugation among the monads.

In *Monas Dallingeri* (*Cercomonas crassicauda*) reproduction occurs by fission of the free-swimming forms and by the conjugation of ameba-like forms, which upon coming in contact immediately coalesce; this "resting stage" persists for about six

hours, when the organism ruptures and liberates multitudes of minute spores, which develop into the adult organism and reproduce by fission as before. In *Oikomonas Dallingeri* certain of the organisms do not proceed to fission, but undergo changes in morphology consisting of the withdrawal of the flagella and great increase in the size of the nucleus; these bodies coming in contact with adult individuals, fuse with them, and become encysted; after from four to five hours the cyst ruptures and very minute spores are liberated which develop into the ordinary dividing forms. In *Heteromita uncinata* the conjugating organisms are differentiated by their size, larger and smaller individuals being seen, the former possessing a contractile vesicle at the anterior end; conjugation consists in the absorption of the smaller form by the larger, followed by encystment, and finally by the rupture of the cyst and the liberation of spores which become the ordinary dividing forms.

I have repeatedly observed what were undoubtedly conjugating forms in *Trichomonas* and *Cercomonas intestinalis* as well as in *Lamblia intestinalis*. In these species conjugation was preceded by the withdrawal or loss of the flagella and the development of irregular or round ameba-like forms; contact of two such organisms resulted in complete fusion and the production of a cystlike body which appeared clear and hyalin. I have not been so fortunate as to observe the rupture of the cyst and the liberation of the young spores.

Pringsheim[30] describes the process of conjugation in *Pandorina* as occurring between two individuals of the swarm-spores, which meet and fuse; the resulting organism encysts and develops within the cyst a swarm-spore, which is liberated when the cyst is placed in a favorable environment; the liberated swarm-spores divide and eventually form the sixteen-celled *Pandorina* colony.

The Sporozoa.—Among the Sporozoa the process of conjugation is highly developed and becomes largely a sexual phenomenon, the male and female organisms being, in most instances, easily distinguishable. However, conjugation in this class of organisms may be very simple, as has already been shown in the description of intracorpuscular conjugation in the malarial plasmodium, where no sex differentiation can be demonstrated.

In the *Gregarinida, Coccidiida,* and *Haemosporidia* conjugation occurs and has been extensively studied by Schaudinn and Siedlecki,[31] Wolters,[32] Grassi, and Feletti,[33] Celli and Sanfelice,[34] MacCallum,[35] and Ross.[36]

Gregarinida.—In this order sporulation is generally preceded by the fission of two gregarines and their encystment. Wolters in *Monocystis agilis* describes the process of conjugation as consisting of the following phenomena: apposition of the end of each organism; mitosis of each nucleus, one portion of each being extruded, the other two daughter-nuclei fusing through an opening in the wall of the organisms at the point of apposition; mitosis of the fused nucleus, one daughter nucleus then going to each conjugant; mitosis of each daughter-nucleus and the formation of spores. This method of conjugation has not been confirmed.

In those instances in which two gregarines unite and become encysted each nucleus reduces, a large portion of it disappears, and, as shown by Siedlecki,[37] a new nucleus, is formed which divides by mitosis; the daughter-nuclei now divide and the division is repeated until the cyst is filled with many nuclei. Each nucleus is surrounded by a portion of the protoplasm and thus *gametes* are formed which use and produce *sporozoites* or the infective organism.

Coccidiida.—Schuberg[38] was the first to suggest conjugation in the *Coccidiida* and Schaudinn and Siedlecki the first to demonstrate that this process is a developmental necessity in these organisms, is a true sexual phenomenon, and results in the fertilization of the female organism. In the *Coccidiida* sexual dimorphism exists, and an alternation of generations occurs, one asexual and the other sexual. In the asexual cycle the coccidia are intracellular in the host, where they sporulate, and the *merozoites*, leaving the host cells, invade new ones; in the sexual cycle conjugation occurs between specialized organisms, the male, or *microgamete*, and the female, or *macrogamete*, and sporogeny follows. Conjugation occurs within the infected individual, but is extracellular.

Hemosporidia.—It is now well known that sexual conjugation occurs among the *Hemosporidia* of both animals and man. The plasmodia of malaria, for example, undergo an asexual cycle of development in the blood of man and a sexual cycle in the body of the mosquito. In the latter, conjugation is absolutely essential to sporulation, but here, in contradistinction to the intracorpuscular conjugation which occurs in the blood of man, the conjugating organisms are sexually differentiated, and in addition intracorpuscular conjugation is not essential to sporulation, but only to the continuation of the infection. In the mosquito conjugation is a fertilizing process *per se*, while intracorpuscular conjugation simply stimulates the organism to renewed activity; the first is a true sexual process, the latter an asexual one, and thus in the two cycles of the malarial plasmodia conjugation is illustrated in its simplest and most highly developed phases.

As conjugation of the malarial plasmodia in the mosquito is so well known I shall not describe it further.

In *Lankestrella* and *Karyolysus*, hemosporidian forms inhabiting cold-blooded vertebrates, Labbe[39] has described a process of conjugation which approaches very closely to intracorpuscular conjugation in the malarial plasmodia. A *trophozoite* after developing for a while in a blood corpuscle becomes free and conjugates with a similar form; the *zygote* thus produced may penetrate a second blood corpuscle or may invade a cell of the spleen, kidney, or bone-marrow, where it becomes encysted; in the cyst are developed *sporozoites* which are liberated when conditions are favorable.

The Infusoria.—It was in this class of the Protozoa that conjugation was first correctly interpreted by Bütschli[40] and Englemann,[41] and here it occurs almost universally. In no other class of the Protozoa is the truth of the statement that the process is one of "rejuvenation" so well illustrated. Reproduction normally occurs in this class by binary division, or less often by multiple division after encystment; whatever the method, reproductive activity finally ceases and conjugation then occurs, restoring the vital activities of the cells by bringing about a regeneration of the protoplasm, and especially of the nucleus. Maupas[42] and Hertwig,[43] as well as Bütschli, have demonstrated that when conjugation occurs, the micronucleus and the macronucleus become separated. The micronucleus divides twice, forming four daughter-nuclei in each of the conjugating individuals; of these eight daughter-nuclei, six degenerate, while the remaining two fuse, the macronucleus meanwhile undergoing complete degeneration. After the formation of the new nucleus it divides and a portion of it forms a new macronucleus. As a rule the conjugating organisms separate, but the union may be permanent.

In *Paramecium aurelia*, Balbiani[44] demonstrated that conjugation occurred after

a series of generations had been evolved by ordinary transverse division, and that it lasted for five or six days, during which time important changes occurred within the conjugants, which finally separated and gave rise to individuals capable of developing as usual.

Gourvitch,[45] Strong,[46] and others have described conjugation as occurring in *Balantidium coli*. Gourvitch states that the conjugating pairs unite and form oval cysts, but his work has not been confirmed and it is probable that the process described by Strong, i. e., the simple fusion of adult organisms is what really occurs. In all probability, after a certain period of time, the conjugants separate and reproduce as before.

General significance of the process of conjugation in the Protozoa.— The process of conjugation in the Protozoa was first observed and described by O. F. Müller.[47] His work was confirmed by Balbiani, who claimed that certain of the Protozoa not only reproduced by simple division but also by conjugation, the latter being a sexual act leading to the formation of the young parasites. It is to Bütschli that we owe the correct interpretation of conjugation in the Protozoa. He observed that continued reproduction of many of these organisms by simple division led eventually to the exhaustion of the capability of division, and thus to the death of the organisms. He, therefore, regarded the process of conjugation as intended to bring about rejuvenescence of the nearly exhausted individuals of a generation of organisms. Englemann confirmed the interpretation of Bütschli, and thus defines the process: "The conjugation of the infusoria does not lead to reproduction through 'eggs,' 'embryonic spores,' or any other kind of germ, but to a peculiar developmental process in the conjugating individual, which may be designated as reorganization." In other words conjugation is intended to bring about a restoration to former reproductive activity, this result being secured by a rejuvenescence of the vital activities of the organism.

Calkins,[48] to whose admirable work I am indebted for many of the references in this contribution, in his discussion of the subject says:

If, as Minot suggested, every newly born organism be regarded as having a certain initial potential energy which is gradually used up in its life-activities to be restored by conjugation, then the union of two cells may be interpreted as a renewal of vigor or a rejuvenescence. . . . . The force of these views as to the need of conjugation for different species of infusoria, at least, can hardly be questioned, for, as repeatedly stated in the previous chapters, reproduction by simple division may go on for a certain number of generations, but cannot continue indefinitely, unless at certain intervals, which Maupas

has shown to be more or less definite, two individuals unite in conjugation. This union, in some wholly unexplained way, imparts to each of the conjugants a renewed vitality, or, in Bütschli's words, a renewal of youth, expressed by increased activity in movements and reproduction. Conjugation thus, as R. Hertwig insists, is not the beginning of a series of reproductive acts, but occurs at or near the end of such a series. . . . . The phenomena of the so-called sexual reproduction and sex differentiation have, in all probability, grown out of this apparently fundamental requirement of living protoplasm, namely, the periodic union of two cells.

Again he says:

The various conjugation phenomena seen in the Protozoa seem to show that each cycle starts with a certain potential of vitality which is gradually exhausted in the vegetative activities of the long line of individuals formed by simple division, or by spore formation.

That conjugation is not a reproductive act in many of the Protozoa is shown by the fact that the time consumed in conjugation is sufficient for reproduction by simple division to occur many times; this is well illustrated in those Protozoa in which a resting stage succeeds conjugation.

While fertilization is generally the result of conjugation it does not follow that the act is a reproductive one, for, as has been pointed out by Hertwig, "a reproductive process is bound up with the encystment of *Actinosphaerium*, whereby the mother-cyst gives rise to many primary cysts, each primary cyst to a germ-sphere, each germ-sphere to new individuals. Reproduction here precedes fertilization and the latter has no effect upon the former."

In reviewing the phenomena of conjugation in the Protozoa it has been shown that in many of them, after the union of the conjugants, a resting or *zygote* stage succeeded, in which the organisms possessed greater resistance to injurious influences, and in which they remained latent, so to speak, and no further development occurred until conditions were favorable. This is true in almost all the lowly forms of plant life in which conjugation has been observed and in many of the Protozoa. A most typical example is found in *Pandorina morum*, in which the *gametes* conjugate and assume a resting form, which is encysted; under favorable conditions the cyst ruptures and one or more individuals emerge and, dividing, in time the typical colony is again formed.

The conjugation of a protozoan within the body of its intermediate host is purely sexual in its nature and is followed at once by repro-

duction. Such a type of conjugation should be clearly distinguished from that in which there is a union of two individuals followed by a period of inactivity or a *zygote* stage. The sexual type occurring within an intermediate host is well illustrated by the union of the *macrogametes* and *microgametocytes* of the malarial plasmodia within the mosquito, while intracorpuscular conjugation of the same organisms is a typical example of asexual conjugation. It is apparent, therefore, that asexual conjugation is not a reproductive act, but one intended to preserve the function of reproduction in a race threatened with extinction by repeated division, or, perhaps, intended to evolve a resistant form when conditions are unfavorable for reproduction in the ordinary way. Again to quote Calkins:

> From all the facts shown at the present time, the only conclusion that can be drawn is that conjugation, apparently, is not the cause of reproduction, but as Butschli, Englemann, and Minot long since pointed out, in some unknown way provides the energy for continuing the functions of the individual, including the power of reproducing.

Significance of intracorpuscular conjugation and its relation to latent and recurrent malarial infections.—Having thus briefly reviewed the process of conjugation and its significance as it occurs in Protozoa in general, we are in a position to consider the significance of that peculiar form of conjugation in the malarial plasmodia which I have called intracorpuscular conjugation. The conjugation of malarial plasmodia within the red-blood cell is asexual, it being impossible to detect any constant difference in the appearance of the two conjugants. It occurs between two young amebula and is completed, so far as can be seen, by the permanent union of both nucleus and protoplasm. The process may be observed in all acute initial infections and in recurrent infections, but only in latent infections, in the peripheral blood, just before the appearance of clinical symptoms.

Continued study of this subject has confirmed the belief I have already expressed, that the process is intended to maintain the malarial infection in the blood of man, and that it occurs whenever the races of plasmodia are in danger of dying out from repeated sporulation in the usual manner; it is therefore most frequently observed in the latter part of an acute attack, instead of before the appearance of clinical symptoms. I am also convinced that in this process there

lies, in all probability, the explanation of latent and recurrent malarial infections.

As has been shown, conjugation occurs in many, if not almost all, organisms, when unfavorable conditions arise, such as exhaustion from repeated division, insufficient nutriment, or the presence of conditions in the environment that are unfavorable to growth in the usual manner; it has also been shown that under such conditions a resting or *zygote* stage succeeds conjugation, in which the usual vital activities are wholly or in part suspended until the conditions again become favorable, when the vatal activities are resumed and reproduction occurs as before.

If we consider carefully the phenomena of intracorpuscular conjugation as seen in the malarial plasmodia it is evident that they conform to those observed during conjugation in many of the Protozoa, and that, if there is any value in analogy, the conditions leading to the process and its significance are similar. Considered in this way I believe that intracorpuscular conjugation is most easily explained, and that the theory of the etiology of latency and recurrence which follows is one that is worthy of careful study and one that is well supported by the known significance of conjugation in other protozoan organisms. Intracorpuscular conjugation in malaria occurs after a series of reproductions by spore-formations, during which time the initial potential energy of the race of plasmodia has gradually declined; during this same period the clinical symptoms of malaria have been present, and the environment of the plasmodia rendered unfavorable, perhaps, by the administration of drugs, such as quinine; as a consequence, decreased ability to reproduce by spore formation leads to intracorpuscular conjugation, and the formation of a resistant form of the plasmodium; the process occurs within the red blood corpuscle, because only here can nutriment be obtained for further development.

When conjugation is completed by the permanent union of the protoplasm and nucleus of the two conjugants, growth occurs at the expense of the red cell, until finally the entire cell is destroyed, and the round, pigmented organism is liberated; this form now, in all probability, becomes encysted and enters upon a resting or *zygote* stage, and it is my belief that it is this stage that has been

considered by Schaudinn[49] as a *macrogamete*, which by parthenogenesis gives rise to recurrences.

This stage is probably more resistant to injurious influences, such as quinine, than other forms of the plasmodia, and may continue unharmed in one of the internal organs, as in the spleen, or, more probably, in the bone marrow, for long periods of time. When conditions are favorable the cyst (for it is probable that the organisms *are* encysted) ruptures, and liberates a generation of spores which have developed within it; these young plasmodia penetrate the red blood corpuscles and reproduce, as is usual, by spore formation. Latency is thus rendered possible by the resistance of the resting or *zygote* stage, and recurrences are due to the liberation and subsequent sporulation of the young plasmodia. In cases in which this form of conjugation is present, numerous large pigmented plasmodia are observed, both within and external to the red cells, which show no evidence at any time of segmentation or flagellation; in these the pigment is small in amount and distributed irregularly in very fine granules; they are often very numerous in the blood of cases in which intracorpuscular conjugation is present and are not observed in the blood of other cases; in aestivo-autumnal infections these bodies are only seen in the blood obtained by splenic puncture. These bodies are probably the ones concerned in the production of the latent forms and eventually become encysted and situated in some internal organ or in the bone marrow.

It would seem that the growth of the conjugating form within the red cell is rapid until the destruction of the cell, when the plasmodium is liberated and enters upon the resting or *zygote* stage; the duration of this stage probably varies with conditions, but I believe that it lasts for several days at least.

Reasoning from analogy this stage must continue for some time, for, as has been shown from the Protozoa, by Bütschli, Maupas, and Hertwig, the two conjugating individuals might by simple division give rise to many others during the time occupied in conjugation, and this is true of every organism in which asexual conjugation occurs, so far as I know It would, indeed, be strange if the malarial plasmodia were exceptions to so general a rule, and therefore it follows that the resting or *zygote* stage must continue for several days, and that

intracorpuscular conjugation, admitting that it produces this form of the plasmodia, is the cause of latency and recurrences. The periods of time between relapses, which vary somewhat, but are remarkably uniform when quinine has not been administered, are explained by the time consumed in the completion of the process of intracorpuscular conjugation and the development of the young plasmodia within the encysted *zygote*, while the marked irregularity in the period between relapses after quinine has been administered, is explained by the liberation of the young plasmodia only when the environment is favorable, i. e., when the quinine has been discontinued, or the quantity administered greatly diminished. We thus see relapses quickly follow the discontinuance of quinine in all malarial regions, and Celli, in his suggestion that relapses may depend upon asexual forms which remain inert in some of the viscera, stated what is in all probability the true explanation of recurrences in malaria, the inert asexual bodies of Celli being the *zygote* or resting form of the plasmodia, produced by intracorpuscular conjugation. That such a latent or resting form of the parasites of malaria is present somewhere in the body in malarial infection is proven by the fact that the withdrawal of quinine in cases which have been taking it for weeks is often followed by a relapse and the reappearance of the parasite in the blood; it is impossible to believe that normal schizogony has occurred in suchcases for weeks, even when quinine has been administered in large doses, without producing symptoms, and while, as I have shown,[50] in discussing the pathology of latent malarial infection, schizogony does occur within the spleen without symptoms being produced, it should be remembered that in the cases in which this was observed no quinine was being administered, and symptoms would probably have soon appeared if the patients had lived. The administration of quinine, even in very moderate doses, has a very marked effect upon the process of intracorpuscular conjugation as observed in the peripheral blood, the conjugating parasites disappearing, perhaps collecting in some internal organ, while in those cases in which the process has not appeared it is never observed if quinine be administered. The large pigmented forms which I believe result from conjugation, show no morphological changes after the administration of quinine.

From my studies of this subject I believe that the following conclusions are justified regarding the significance of intracorpuscular conjugation:

1. Intracorpuscular conjugation is the chief cause of the maintenance of malarial infection.

2. It maintains malarial infection by producing a resting or *zygote* stage of the plasmodia, within the human body, which is resistant to quinine and other injurious influences.

3. It is the cause of latency and recurrences of malarial infection, the *zygote* stage remaining dormant or "latent" until conditions are favorable, when it gives birth to several young plasmodia, thus causing a recurrence of the infection.

These conclusions are justified by the following considerations:

1. The presence of the process in all acute and recurrent infections.

2. The fact that during the time consumed in conjugation in all other Protozoa, provided the conjugation is asexual, many generations of the organism could have been produced by division or sporulation in the usual manner.

3. The fact that such a resting or *zygote* stage must exist, as proven by the recurrence of the infection after the discontinuance of quinine given for long periods of time.

4. The fact that in cases which have been treated at once with sufficient doses of quinine and for a sufficiently long period, intracorpuscular conjugation is never seen, and in such cases relapses are very rare, if they ever occur.

5. The presence of numerous large pigmented bodies in the blood in cases in which the process is most marked, both intra- and extracellular, and which are not seen in cases in which the process is absent.

6. The argument from "analogy" which indicates that the significance of the process of asexual conjugatoin in the malarial plasmodia is similar to the same process in other of the Protozoa.

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EXPLANATION OF PLATE 2.

A. SCHIZOGONY OF MALARIAL PLASMODIUM. ACTIVE CYCLE.

1 to 7.—Active cycle of *schizogony*, showing development of the *merozoite* into *schizont* after penetrating the red-blood corpuscle, and the development and final sporulation of the *schizont*.

B. SCHIZOGONY OF MALARIAL PLASMODIUM. LATENT CYCLE.

1^a *to* 9^a.—Latent cycle of *schizogony*. Two of the *merozoites* (6) enter a red blood corpuscle and there conjugate (1^a to 6^a), producing a single organism which grows until the red-blood corpuscle is destroyed, when it is liberated and eventually becomes encysted, developing within the cyst several young plasmodia (7^a) which, when conditions are favorable, are liberated by the rupture of the cyst (8^a) and, penetrating the red corpuscles, undergo development as in the active cycle of the *schizogony*.

C. SPOROGONY OF MALARIAL PLASMODIUM.

1^b *to* 21^b.—Illustrating steps in the *sporogony* of the malarial plasmodium within the mosquito.

1^b *to* 6^b.—Development of the *macrogamete* and the *microgametocyte* which occurs within man.

7^b *and* 8^b.—Development of the *macrogamete* and *microgamete* (free flagellum) within the stomach of the mosquito.

9^b.—Fertilization of the *macrogamete* by the *microgamete*.

10^b *to* 17^b.—Development of the *oökinete*, *oöcyst*, *sporoblast*, and *sporozoites*.

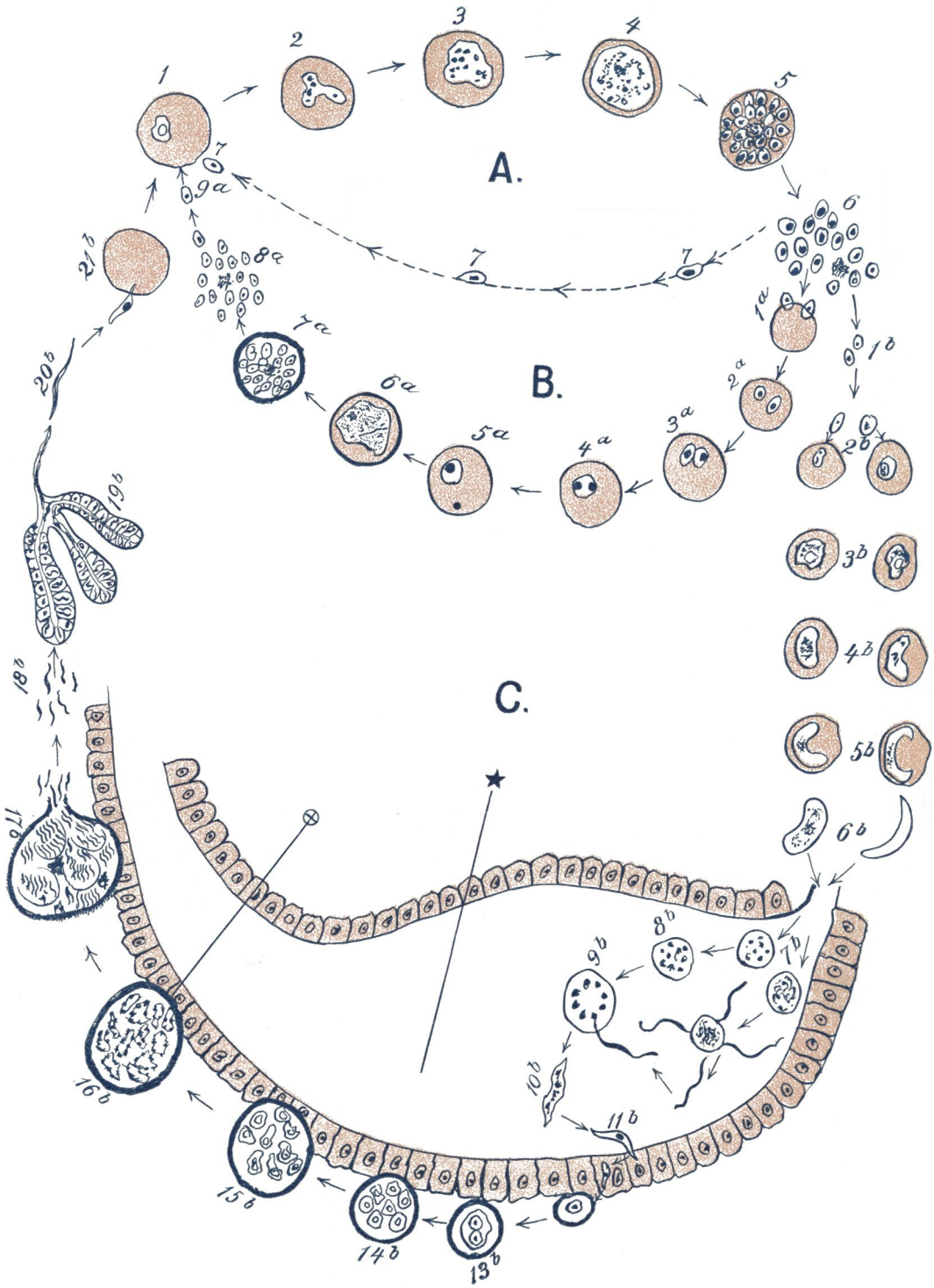
18^b.—Liberated *sporozoites*, which reach the salivary gland of the mosquito (19^a).

20^b *and* 21^b.—*Sporozoite* passing from salivary gland and eventually penetrating the red blood corpuscle of man.

*—Interior of stomach of mosquito.

†—Wall of stomach of mosquito.

PLATE 2.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